Cell Biology

CELL SWELLING STIMULATES A RISE IN INTRACELLULAR CALCIUM IN *NECTURUS* ERYTHROCYTES. Andrew J. Attwood and Douglas B. Light*. Department of Biology, Ripon College, Ripon, WI 54971 (attwooda@ripon.edu and lightd@ripon.edu).

The ability of animal cells to regulate volume is a fundamental property common to a large number of cell types and is evolutionarily one of the oldest regulatory mechanisms. Volume regulation is of importance in cells exposed to anisotonic extracellular media and in cells where transport of solutes change intracellular osmolality. The purpose of this study was to examine the role of Ca^{2+}, a ubiquitous intracellular messenger, in regulated volume decrease by *Necturus maculosus* erythrocytes. An increase in intracellular Ca^{2+} was determined with epifluorescence microscopy and the Ca^{2+}-sensitive dye Fluo-4-AM (10 μM), which glows in the presence of this ion. Exposure to hypotonic (0.5X) amphibian Ringer stimulated an increase in intracellular Ca^{2+} levels, as indicated by an increase in fluorescence. In contrast, a rise in Ca^{2+} did not occur under isosmotic conditions, unless cells were exposed to the Ca^{2+} ionophore A23187 (2 μM), which increased fluorescence similar to that of swollen cells. Interestingly, a 0 Ca^{2+}-EGTA Ringer only slightly inhibited fluorescence in swollen cells, suggesting that the main source of Ca^{2+} during cell swelling was from intracellular stores. In addition, gadolinium (10 μM, an antagonist of stretch-activated channels), hexokinase (2.5 U/ml, an enzyme that dephosphorylates ATP in the presence of glucose), and suramin (100 μM, a general purinoceptor antagonist) each inhibited the swelling induced increase in calcium. The inhibitory effect of all three of these agents was reversed with A23187. Conclusions: cell swelling stimulated an increase in intracellular Ca^{2+}. Our results suggest that the main source of this ion was from intracellular stores. Further, an increase in cytosolic calcium during hypotonic shock was linked to extracellular ATP activation of a purinoceptor. (Funded by NSF grant MCB-0076006).